

Claims

1. A method for producing a zinc finger nucleic acid binding protein comprising preparing a zinc finger protein according design rules, varying the protein at one or more positions, and selecting variants which bind to a target nucleic acid sequence by polysome display.
2. A method according to claim 1, wherein the protein is varied at one or more positions selected from the group consisting of +1, +5, +8, -1, +2, +3 and +6.
3. A method for producing a zinc finger nucleic acid binding protein comprising an at least partially varied sequence and selecting variants thereof which bind to a target DNA strand, comprising the steps of:
 - (i) preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class capable of binding to a nucleic acid triplet in a target nucleic acid sequence, wherein binding to each base of the triplet by an α -helical zinc finger nucleic acid binding motif in the protein is determined as follows:
 - a) if the 5' base in the triplet is G, then position +6 in the α -helix is Arg; or position +6 is Ser or Thr and position ++2 is Asp;
 - b) if the 5' base in the triplet is A, then position +6 in the α -helix is Gln and ++2 is not Asp;
 - c) if the 5' base in the triplet is T, then position +6 in the α -helix is Ser or Thr and position ++2 is Asp;
 - d) if the 5' base in the triplet is C, then position +6 in the α -helix may be any amino acid, provided that position ++2 in the α -helix is not Asp;
 - e) if the central base in the triplet is G, then position +3 in the α -helix is His;
 - f) if the central base in the triplet is A, then position +3 in the α -helix is Asn;
 - g) if the central base in the triplet is T, then position +3 in the α -helix is Ala, Ser or Val; provided that if it is Ala, then one of the residues at -1 or +6 is a small residue;

h) if the central base in the triplet is C, then position +3 in the α -helix is Ser, Asp, Glu, Leu, Thr or Val;

i) if the 3' base in the triplet is G, then position -1 in the α -helix is Arg;

j) if the 3' base in the triplet is A, then position -1 in the α -helix is Gln;

5 k) if the 3' base in the triplet is T, then position -1 in the α -helix is Asn or Gln;

l) if the 3' base in the triplet is C, then position -1 in the α -helix is Asp;

(ii) varying the resultant polypeptide at at least one position; and

10 (iii) selecting the variants which bind to a target nucleic acid sequence by polysome display.

4. A method according to any preceding claim, wherein the or each zinc finger has the general primary structure

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(A) X^a C X_{2-4} C X_{2-3} F X^c X X X X L X X H X X X^b H - linker
-1 1 2 3 4 5 6 7 8 9

wherein X (including X^a , X^b and X^c) is any amino acid.

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5. A method according to claim 5 wherein X^a is $F/Y-X$ or $P-F/Y-X$.

6. A method according to claim 4 or claim 5 wherein X_{2-4} is selected from any one of: S-X, E-X, K-X, T-X, P-X and R-X.

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7. A method according to any one of claims 4 to 6 wherein X^b is T or I.

8. A method according to any one of claims 4 to 7 wherein X_{2-3} is G-K-A, G-K-C, G-K-S, G-K-G, M-R-N or M-R.

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9. A method according to any one of claims 4 to 8 wherein the linker is T-G-E-K or T-G-E-K-P.

10. A method according to any one of claims 4 to 9 wherein position +9 is R or K.
11. A method according to any one of claims 4 to 10 wherein positions +1, +5 and +8
5 are not occupied by any one of the hydrophobic amino acids, F, W or Y.
12. A method according to claim 11 wherein positions +1, +5 and +8 are occupied by the residues K, T and Q respectively.
- 10 13. A method according to any preceding claim, wherein the polysome display technique comprises the steps of:
- (a) introducing a population of mRNA species into an in vitro translation system under conditions suitable for translation to form a pool of polysomes displaying nascent
15 zinc finger polypeptides;
- (b) contacting the polysomes with a target nucleic acid under suitable binding conditions;
- (c) selecting polysomes which are specifically bound to the nucleic acid; and
- (d) reverse transcribing and amplifying the isolated mRNA.